UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

| APPLICATION NO. | FILING DATE | FIRST NAMED INVENTOR | ATTORNEY DOCKET NO. | CONFIRMATION NO. |
|---|---|---|---|---|
| 10/759,179 | 01/20/2004 | Chihiro Uematsu | 1021.43414X00 | 7828 |

20457 7590 07/17/2006

ANTONELLI, TERRY, STOUT & KRAUS, LLP
1300 NORTH SEVENTEENTH STREET
SUITE 1800
ARLINGTON, VA 22209-3873

| EXAMINER | |
|---|---|
| MUMMERT, STEPHANIE KANE | |
| **ART UNIT** | **PAPER NUMBER** |
| 1637 | |

DATE MAILED: 07/17/2006

Please find below and/or attached an Office communication concerning this application or proceeding.

| Office Action Summary | Application No. | Applicant(s) | |
|---|---|---|---|
| | 10/759,179 | UEMATSU ET AL. | |
| | Examiner | Art Unit | |
| | Stephanie K. Mummert, Ph.D. | 1637 | |

*-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --*

**Period for Reply**

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

**Status**

1)☐ Responsive to communication(s) filed on *29 March 2006*.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

**Disposition of Claims**

4)☒ Claim(s) *1-9 and 14* is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1-9 and 14* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

**Application Papers**

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

**Priority under 35 U.S.C. § 119**

12)☒ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☒ All b)☐ Some * c)☐ None of:

1.☒ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

**Attachment(s)**

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☐ Information Disclosure Statement(s) (PTO-1449 or PTO/SB/08) Paper No(s)/Mail Date _____.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____.
5) ☐ Notice of Informal Patent Application (PTO-152)
6) ☐ Other: _____.

## SUPPLEMENTAL ACTION

**This supplemental action is sent to correct the previous office action, mailed June 13, 2006. The supplemental action includes the correct citation to Wenz (US PgPub 2003/0190646; October 2003) which was omitted from the previously mailed action.**

Applicant's amendment filed on March 29, 2006 is acknowledged and has been entered. Claims 1-2 have been amended. Claim 14 has been added. Claims 1-9 and 14 are pending.

Claims 1-9 and 14 are discussed in this Office action.

Applicant's arguments with respect to claims 1-9 have been considered but are moot in view of the new ground(s) of rejection. Any rejection not reiterated in this action has been withdrawn as being obviated by the amendment of the claims.

***Previous Rejections***

The objection to claim 4 under 37 CFR 1.75(c) as being of improper dependent form for failing to further limit the subject matter of a previous claim is withdrawn in view of Applicant's arguments. The current rejection of claims 1-3 under 35 U.S.C. 112, second paragraph is withdrawn in view of Applicant's arguments.

***NEW GROUNDS OF REJECTION***

***Claim Rejections - 35 USC § 103***

1. The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

2. Claims 1-9 are rejected under 35 U.S.C. 103(a) as being unpatentable over Wenz et al. (US PgPub 2003/0190646; October 2003) in view of Ovyn et al. (6,1101,681; August 2000), Livak et al. (US Patent 5,538,848; July 1996) and further in view of Eun et al. (2000, Journal of Virological Methods, vol. 87, p. 151-160). Ovyn teaches a method of amplification that incorporates the NASBA or nucleic acid sequence based amplification system for the detection of variants of Mycoplasma pneumonia (col. 1, lines 19 to col. 3, line 15).

With regard to claim 1, Wenz teaches a method for expressed gene analysis, which comprises the steps of: A) subjecting a gene to be analyzed to nucleic acid amplification using 1) a forward primer specifically hybridizing to the gene to be analyzed (Figures 1-3), 2) a primer for introduction comprising a first base sequence closer to the 5' end of the primer than a third base sequence comprising a sequence specifically hybridizing to a target gene (Figure 1-3, p. 3, paragraph 28-30, where the probe/primer has a portion that is complementary to the target, T-SP, a portion that is complementary to a secondary primer, P-SP and a portion that comprises an addressable-support specific portion, AS-SP that is located between the target-specific and primer-specific portion); 3) a probe comprising a base sequence identical or complementary to the first base sequence and labeled at one end with a fluorophore (Figure 1-3, p. 3, paragraph 28-30, where the probe/primer has a portion that is complementary to the target, T-SP, a portion that

is complementary to a secondary primer, P-SP and a portion that comprises an addressable-support specific portion, AS-SP that is located between the target-specific and primer-specific portion; see p. 1, paragraph 10, where at least one primer comprises a reporter group), and

C) detecting fluorescence emitted by the released fluorophore, thereby assaying the amount of the product of the nucleic acid amplification (Figure 3, step K, where the amplified product is bound by a probe attached to an addressable array),

Regarding claim 1, Wenz does not teach that the primer for introduction comprises a second base sequence comprising a promoter sequence of RNA polymerase, which is non-specific to the base sequence of the target gene. Wenz also does not teach that the amplification of the gene is accomplished using reverse transcriptase, RNA polymerase and ribonuclease H and/or exonuclease.

With regard to claim 1, Ovyn teaches a method for expressed gene analysis, which comprises the steps of: A) subjecting a gene to be analyzed to nucleic acid amplification using 2) a primer for introduction comprising a second base sequence closer to the 5' end of the primer than the first base sequence (col. 4, lines 48-62, where the primer may include a promoter sequence) 4) reverse transcriptase (col. 2, lines 65-66; see also Figure 1, where the reverse transcriptase is AMV-RT), 5) RNA polymerase (col. 2, lines 65-66, where the RNA polymerase is T7 RNA polymerase, see also Figure 1), and 6) ribonuclease H and/or exonuclease (col. 2, lines 65-66, see Figure 1); and

C) detecting fluorescence emitted by the released fluorophore, thereby assaying the amount of the product of the nucleic acid amplification (col. 9, lines 45-55, where detection probes were hybridized to horseradish peroxidase and the amount of HRP conjugated oligonucleotides was

measured to detect target sequence; however as noted at col. 6, lines 30-37, the probe may also be labeled by fluorescent moieties),

wherein the gene to be analyzed is prepared by the introduction of the first base sequence and the second base sequence comprising a promoter sequence of RNA polymerase, which are non-specific to the base sequence of the target gene, into the target gene so that the second base sequence is bound to a position closer to the 5' end of the gene to be analyzed than the first base sequence (see Figure 1 and description recited above).

With regard to claim 2, Ovyn teaches an embodiment of claim 1, wherein the gene to be analyzed is cDNA comprising the first base sequence and the second base sequence introduced therein by the introduction with subjecting mRNA of the target gene to reverse transcription using the primer for introduction which comprises the first base sequence as described for claim 1 above, in step A) 2) (col. 5, lines 46-64, where primer P1 hybridizes to the RNA to prime and initiate 1$^{st}$ strand synthesis and where after second strand synthesis, the complete cDNA includes the T7 promoter site from the P1 primer).

With regard to claims 3 and 4, Ovyn teaches an embodiment of claim 1, wherein the nucleic acid amplification is conducted by sequentially repeating the following steps 1) to 3):

1) transcription of the gene to analyzed into RNA with the aid of RNA polymerase (col. 5, lines 46-67, see also Figure 1);

2) reverse transcription of the RNA using the forward primer and the reverse transcriptase or ribonuclease H to synthesize single-stranded cDNA (see Figure 1); and

3) synthesis of the gene to be analyzed from the single-stranded cDNA using the primer for introduction and DNA polymerase (col. 5, lines 46-67 and Figure 1).

With regard to claim 5, Ovyn teaches an embodiment of claim 1, wherein the nucleic acid amplification is conducted at a substantially single temperature (col. 10, lines 15-25, where isothermal amplification of the target RNA was performed at 41°C).

With regard to claim 6, Ovyn teaches an embodiment of claim 5, wherein the single temperature is between 37°C and 55°C (col. 10, lines 15-25, where isothermal amplification of the target RNA was performed at 41°C and wherein 41°C is between 37°C and 55°C).

With regard to claim 7, Ovyn teaches an embodiment of claim 1, wherein the RNA polymerase is T7 RNA polymerase and the second base sequence comprises the T7 promoter sequence (col. 5, lines 46-64, where primer P1 hybridizes to the RNA to prime and initiate 1st strand synthesis and where after second strand synthesis, the complete cDNA includes the T7 promoter site from the P1 primer; see also Figure 1).

Ovyn does not teach that the probe for detection of the amplified nucleic acids is labeled at one end with a fluorphore and at the other end with a quencher or the digestion of the probe bound to the first base sequence by the ribonuclease H or exonuclease at the time of the nucleic acid amplification. Livak teaches the inclusion of a probe labeled at one end with a fluorophore and at the other end with a quencher as part of quantitative Real Time PCR amplification and teaches that the probe bound to the target is digested by an endonuclease (col. 3, lines 49-67).

Neither Ovyn or Livak teach the simultaneous detection of two target genes simultaneously. Eun teaches the simultaneous quantitation of two orchid viruses, cymbidium mosaic potextvirus (CymMV) and odontoglossum ringspot tobamovirus (ORSV) using the TaqMan real-time RT-PCR technique (Abstract, lines 1-3).

With regard to claim 8, Eun teaches an embodiment of claim 1, wherein two or more target genes are simultaneously detected in a single reaction vessel using two or more types of probes (Table 1, where probes directed to CymMV and ORSV are described).

With regard to claim 9, Eun teaches an embodiment of claim 8, wherein the melting temperatures (Tm values) of the two or more types of probes are substantially the same (Table 2, where the probes have Tm values of 69, 68 and 70, for CymMV RdRp, CymMV CP, ORSV RdRp, ORSV CP respectively).

It would have been prima facie obvious to one of ordinary skill in the art at the time the invention was made to incorporate the primer formulation, including a target-specific component and a target non-specific component specific to the probe sequence taught by Wenz into the method of NASBA amplification taught by Ovyn to arrive at the claimed invention with a reasonable expectation for success. The inclusion of a third primer portion, comprising an RNA promoter sequence, would have also been an obvious and necessary substitution to the three-part primer described by Wenz for the use of the primers in a NASBA amplification format. As noted by Wenz, the inclusion of an addressable-array specific portion (which serves the same functional role of detection as the hybridization of a sequence-specific probe, such as the type taught by Livak) "is designed to specifically hybridize with a unique capture oligonucleotide on an addressable support or to have a mobility such that it is located at a particular mobility address during or after appropriate procedures" (p. 10, paragraph 115). Furthermore, since the probes/primers taught by Wenz may be designed to share primer-specific portions (P-SP, second sequence portion) but comprise different addressable support-specific portions, "as few as two or three 'universal' primers can be used to amplify an infinite number of ligation or amplification

products" (p. 12, paragraph 131). While Ovyn and Wenz carry out the amplification and detection components of the inventions in a different manner, both disclose embodiments directed to the amplification of target sequences in a manner that is isothermal (p. 8, paragraph 90 of Wenz).

It would have been prima facie obvious to one of ordinary skill in the art at the time the invention was made to substitute the TaqMan probe for the ECL detection step used by Ovyn. The probe taught by Livak, is labeled at one end with a fluorophore and a quencher at the other end, where fluorescence is detected when the probe is digested through the 5'-3' exonuclease activity of a DNA polymerase. As taught by Livak, it is preferred that the reporter and quencher molecules are attached at the 5' and 3' terminal ends because the probes "are readily synthesized and ameliorate inefficiencies in hybridization and exonuclease cleavage due to groups which are attached to internal bases or internucleotide linkages" because "a quencher molecule need not be attached to a nucleotide adjacent to a reporter molecule to successfully quench fluorescence produced by the reporter when the probe is in a single stranded state (col. 3, lines 49-67)." Furthermore, as noted by Eun, "the development of the TaqMan 5' nuclease assays represents a significant advance in nucleic acid quantification" (p. 157, col. 2) and further notes that "TaqMan real-time RT-PCR has many advantages over conventional PCR that requires post-amplification processing such as agarose gel electrophoresis. Such steps increase the risk of inaccuracy and contamination... No post amplification steps are required and the calculation of the initial amount of starting material is performed automatically by the software program. When coupled with a 96-well capacity, this system offers a sensitive, high-throughput and rapid method for plant virus detection (p. 158, col 2)." The benefit of increased sensitivity and high-

throughput analysis would have been obvious to one of ordinary skill in the art who therefore would have been motivated to incorporate the multiple probes taught by Eun into the NASBA amplification technique taught by Ovyn with a reasonable expectation of success.

3. Claims 1-7 are rejected under 35 U.S.C. 103(a) as being unpatentable over Wenz et al. (US PgPub 2003/0190646; October 2003) in view of Leone et al. (1998, Nucleic Acids Research, vol. 26, no. 9, p. 2150-2155), Bass et al. (US PgPub 2001/0039014; November 2001) and further in view of Mackay et al. (2002, Nucleic Acids Research, vol. 30, no. 6, p. 1292-1305). Leone discloses the use of molecular beacon probes in the detection of nucleic acids amplified by the NASBA technique (Abstract).

With regard to claim 1, Wenz teaches a method for expressed gene analysis, which comprises the steps of: A) subjecting a gene to be analyzed to nucleic acid amplification using 1) a forward primer specifically hybridizing to the gene to be analyzed (Figures 1-3), 2) a primer for introduction comprising a first base sequence closer to the 5' end of the primer than a third base sequence comprising a sequence specifically hybridizing to a target gene (Figure 1-3, p. 3, paragraph 28-30, where the probe/primer has a portion that is complementary to the target, T-SP, a portion that is complementary to a secondary primer, P-SP and a portion that comprises an addressable-support specific portion, AS-SP that is located between the target-specific and primer-specific portion); 3) a probe comprising a base sequence identical or complementary to the first base sequence and labeled at one end with a fluorophore (Figure 1-3, p. 3, paragraph 28-30, where the probe/primer has a portion that is complementary to the target, T-SP, a portion that is complementary to a secondary primer, P-SP and a portion that comprises an addressable-

support specific portion, AS-SP that is located between the target-specific and primer-specific portion; see p. 1, paragraph 10, where at least one primer comprises a reporter group), and C) detecting fluorescence emitted by the released fluorophore, thereby assaying the amount of the product of the nucleic acid amplification (Figure 3, step K, where the amplified product is bound by a probe attached to an addressable array),

Regarding claim 1, Wenz does not teach that the primer for introduction comprises a second base sequence comprising a promoter sequence of RNA polymerase, which is non-specific to the base sequence of the target gene. Wenz also does not teach that the amplification of the gene is accomplished using reverse transcriptase, RNA polymerase and ribonuclease H and/or exonuclease.

With regard to claim 1, Leone teaches a method for expressed gene analysis, which comprises the steps of: A) subjecting a gene to be analyzed to nucleic acid amplification using 1) a forward primer specifically hybridizing to the gene to be analyzed (p. 2151, col. 1, 'selection of amplification primers and probe' heading, where PD415 or PD416 are antisense primers and PD417 is a sense primer, which were designed to amplify the coat protein open reading frame), 2) a primer for introduction comprising a second base sequence closer to the 5' end of the primer than the first base sequence (p. 2154, Figure 6, legend, where it is noted that the amplicon formed by PD415-PD417 or PD416-PD417 contain a binding site for the T7 RNA polymerase, in addition to complementarity to the target sequence, as described in more detail in Leone et al. 1997, J. Virol. Methods, 66, 19-27, see Table 1, where the sequences of PD415-PD417 are given and also the '*2.2 Selection of amplification primers and detection probe*' heading, where "the antisense ones consisted of a 3' terminal target specific sequence and a 5' terminal T7 promoter

sequence"), 3) a probe comprising a base sequence identical or complementary to the first base sequence and labeled at one end with a fluorophore (p. 2151, col. 1, 'synthesis of the molecular beacons' heading, where a molecular beacon sw75-F1 was designed to bind to nucleotides within the coat protein ORF of PLRV, with DABCYL at the 3' end and fluoroscein at the 5' end), 4) reverse transcriptase (p. 2151, 'NASBA' heading, where the reverse transcriptase was included as part of the enzyme mix, which included 6.4 U AMV-reverse transcriptase), 5) RNA polymerase (p. 2151, 'NASBA' heading, where the RNA polymerase is T7 and 32 U are included in the enzyme mix), and 6) ribonuclease H and/or exonuclease (p. 2151, 'NASBA' heading, where 0.08 U RNase H is included in the enzyme mix); and

C) detecting fluorescence emitted by the released fluorophore, thereby assaying the amount of the product of the nucleic acid amplification (p. 2151, col. 2 'post-NASBA analysis' and 'Real-time monitoring of NASBA reactions and thermal denaturation profiles' heading, see also Figure 2), wherein the gene to be analyzed is prepared by the introduction of the first base sequence and the second base sequence comprising a promoter sequence of RNA polymerase, which are non-specific to the base sequence of the target gene, into the target gene so that the second base sequence is bound to a position closer to the 5' end of the gene to be analyzed than the first base sequence.

With regard to claim 2, Leone teaches an embodiment of claim 1, wherein the gene to be analyzed is cDNA comprising the first base sequence and the second base sequence introduced therein by the introduction with subjecting mRNA of the target gene to reverse transcription using a primer for introduction which comprises the first base sequence as described for claim 1 above, in step A) 2) (see description above for Step A) 2), also p. 2150, col. 1, where the process

of NASBA is described in more detail, where the reaction is based on the concurrent activity of AMV-RT, RNase H and T7 polymerase).

With regard to claims 3 and 4, Leone teaches an embodiment of claim 1, wherein the nucleic acid amplification is conducted by sequentially repeating the following steps 1) to 3):
1) transcription of the gene to analyzed into RNA with the aid of RNA polymerase;
2) reverse transcription of the RNA using the forward primer and the reverse transcriptase or ribonuclease H to synthesize single-stranded cDNA; and
3) synthesis of the gene to be analyzed from the single-stranded cDNA using the primer for introduction and DNA polymerase (p. 2150, col. 1, where the process of NASBA is described in more detail, where the reaction is based on the concurrent activity of AMV-RT, RNase H and T7 polymerase in repetition and where the activity of these enzymes would include each of the preceeding steps recited, including transcription with an RNA polymerase, reverse transcription, and synthesis of the gene using DNA polymerase).

With regard to claim 5, Leone teaches an embodiment of claim 1, wherein the nucleic acid amplification is conducted at a substantially single temperature (p. 2151, 'NASBA' heading, where besides an incubation at 65°C prior to introduction of the enzyme mix, the reaction occurred at 41°C).

With regard to claim 6, Leone teaches an embodiment of claim 5, wherein the single temperature is between 37°C and 55°C (p. 2151, 'NASBA' heading, where besides an incubation at 65°C prior to introduction of the enzyme mix, the reaction occurred at 41°C).

With regard to claim 7, Leone teaches an embodiment of claim 1, wherein the RNA polymerase is T7 RNA polymerase and the second base sequence comprises the T7 promoter

sequence (p. 2154, Figure 6, legend, where it is noted that the amplicon formed by PD415-PD417 or PD416-PD417 contain a binding site for the T7 RNA polymerase, in addition to complementarity to the target sequence, as described in more detail in Leone et al. 1997, J. Virol. Methods, 66, 19-27, see Table 1, where the sequences of PD415-PD417 are given and also the '*2.2 Selection of amplification primers and detection probe*' heading, where "the antisense ones consisted of a 3' terminal target specific sequence and a 5' terminal T7 promoter sequence").

Leone does not teach that the probe for detection of the amplified nucleic acids is labeled at one end with a fluorphore and at the other end with a quencher or the digestion of the probe bound to the first base sequence by the ribonuclease H or exonuclease at the time of the nucleic acid amplification. However, Bass teaches that a molecular beacon probe molecule with a fluorophore at one end and a quencher at the other for the 'real time' detection of reactions amplified via NASBA is an equivalent alternative to the Taqman hydrolysis probe previously described (p. 36, paragraph 0329, lines 1-12).

It would have been prima facie obvious to one of ordinary skill in the art at the time the invention was made to incorporate the primer formulation, including a target-specific component and a target non-specific component specific to the probe sequence taught by Wenz into the method of NASBA amplification taught by Leone to arrive at the claimed invention with a reasonable expectation for success. The inclusion of a third primer portion, comprising an RNA promoter sequence, would have been an obvious and necessary substitution to the three-part primer described by Wenz for the use of the primers in a NASBA amplification format. As noted by Wenz, the inclusion of an addressable-array specific portion (which serves the same functional role as the hybridization of a sequence-specific probe, such as the type taught by

Livak) "is designed to specifically hybridize with a unique capture oligonucleotide on an addressable support or to have a mobility such that it is located at a particular mobility address during or after appropriate procedures" (p. 10, paragraph 115). While Leone and Wenz carry out the amplification and detection components of the inventions in a different manner, both are directed to the amplification of target sequences in a manner that is isothermal (p. 8, paragraph 90 of Wenz).

It would have been prima facie obvious to one of ordinary skill in the art at the time the invention was made to substitute the molecular beacon probe taught by Bass for the TaqMan probe specified in the instant application. The TaqMan probe is labeled at one end with a fluorophore and a quencher at the other end, where fluorescence is detected when the probe is digested through the exonuclease activity of a processive thermostable polymerase. As taught by Bass, "an alternative to TaqMan is the use of molecular beacons to assess library quality. Molecular beacons are hairpin shaped molecules with an internally quenched fluorophore whose fluorescence is restored when they bind to a target nucleic acid" (p. 36, paragraph 0329, lines 1-12). Bass also notes that "when the probe encounters a target molecule, it forms a hybrid that is longer and more stable than the stem hybrid and its rigidity and length preclude the simultaneous existence of the stem hybrid" (p. 36, paragrah 0329, lines 21-25). Furthermore, as noted by Mackey, because a mismatch between a hairpin probe and its target has a destabilizing effect on hybridization "hairpin oligoprobes have been shown to be more specific than the more common linear oligoprobes, making them ideal candidates for detecting SNPs" (p. 1297, col. 2 'hairpin oligoprobes' heading). One of ordinary skill in the art would have recognized the benefit of high specificity of detection of target sequences, who therefore would have been motivated to

substitute the molecular beacon taught by Bass for the Taqman probe alternative for real-time detection with a reasonable expectation for success.

4. Claim 14 is rejected under 35 U.S.C. 103(a) as being unpatentable over Wenz et al. (US PgPub 2003/0190646; October 2003) in view of Ovyn et al. (6,1101,681; August 2000) and further in view of Rizzo et al. (Molecular and Cellular Probes, 2002, vol. 16, p. 277-283). Ovyn teaches a method of amplification that incorporates the NASBA or nucleic acid sequence based amplification system for the detection of variants of Mycoplasma pneumonia (col. 1, lines 19 to col. 3, line 15).

With regard to claim 1, Wenz teaches a method for expressed gene analysis, which comprises the steps of: A) subjecting a gene to be analyzed to nucleic acid amplification using 1) a forward primer specifically hybridizing to the gene to be analyzed (Figures 1-3), 2) a primer for introduction comprising a first base sequence closer to the 5' end of the primer than a third base sequence comprising a sequence specifically hybridizing to a target gene (Figure 1-3, p. 3, paragraph 28-30, where the probe/primer has a portion that is complementary to the target, T-SP, a portion that is complementary to a secondary primer, P-SP and a portion that comprises an addressable-support specific portion, AS-SP that is located between the target-specific and primer-specific portion); 3) a probe comprising a base sequence identical or complementary to the first base sequence and labeled at one end with a fluorophore (Figure 1-3, p. 3, paragraph 28-30, where the probe/primer has a portion that is complementary to the target, T-SP, a portion that is complementary to a secondary primer, P-SP and a portion that comprises an addressable-

support specific portion, AS-SP that is located between the target-specific and primer-specific portion; see p. 1, paragraph 10, where at least one primer comprises a reporter group), and

C) detecting fluorescence emitted by the released fluorophore, thereby assaying the amount of the product of the nucleic acid amplification (Figure 3, step K, where the amplified product is bound by a probe attached to an addressable array).

Regarding claim 1, Wenz does not teach that the primer for introduction comprises a second base sequence comprising a promoter sequence of RNA polymerase, which is non-specific to the base sequence of the target gene. Wenz also does not teach that the amplification of the gene is accomplished using reverse transcriptase, RNA polymerase and ribonuclease H and/or exonuclease.

With regard to claim 1, Ovyn teaches a method for expressed gene analysis, which comprises the steps of: A) subjecting a gene to be analyzed to nucleic acid amplification using 2) a primer for introduction comprising a second base sequence closer to the 5' end of the primer than the first base sequence (col. 4, lines 48-62, where the primer may include a promoter sequence) 4) reverse transcriptase (col. 2, lines 65-66; see also Figure 1, where the reverse transcriptase is AMV-RT), 5) RNA polymerase (col. 2, lines 65-66, where the RNA polymerase is T7 RNA polymerase, see also Figure 1), and 6) ribonuclease H and/or exonuclease (col. 2, lines 65-66, see Figure 1); and

C) detecting fluorescence emitted by the released fluorophore, thereby assaying the amount of the product of the nucleic acid amplification (col. 9, lines 45-55, where detection probes were hybridized to horseradish peroxidase and the amount of HRP conjugated oligonucleotides was

measured to detect target sequence; however as noted at col. 6, lines 30-37, the probe may also be labeled by fluorescent moieties),

wherein the gene to be analyzed is prepared by the introduction of the first base sequence and the second base sequence comprising a promoter sequence of RNA polymerase, which are non-specific to the base sequence of the target gene, into the target gene so that the second base sequence is bound to a position closer to the 5' end of the gene to be analyzed than the first base sequence (see Figure 1 and description recited above).

Neither Ovyn or Wenz teach that the probe for detection of the amplified nucleic acids is labeled at one end with a fluorphore and at the other end with a quencher or the digestion of the probe bound to the first base sequence by the ribonuclease H or exonuclease at the time of the nucleic acid amplification. Rizzo teaches that the probe for detection is labeled at one end with a quencher and that the probe is digested by ribonuclease H (Figure 1, where the probe is a molecular beacon and is labeled at one end with a quencher and at the other end with a fluorophore).

Regarding claim 14, Rizzo teaches an embodiment of claim 1, wherein the probe is a DNA/RNA hybrid (Figure 1, where the probe is comprised of an RNA:DNA hybrid stem).

It would have been *prima facie* obvious to one of ordinary skill in the art at the time the invention was made to have modified the method of expressed gene analysis taught by Ovyn to incorporate an RNA/DNA hybrid probe of the type disclosed by Rizzo. Rizzo states that "here we describe the preparation of an RNA/DNA chimeric molecular beacon, which contains a single-stranded RNA-DNA chimeric oligonucleotide labeled with a 5' fluorescein as fluorophore and a 3'-DABCYL as quencher (Figure 1). The fluorophore of the probe is held in proximity to

the quencher by the stem-loop structure. When the RNA sequence of the RNA:DNA hybrid stem is cleaved, the fluorescence of the fluorophore is manifested." (p. 278, col. 1, 2 paragraph). Furthermore, Rizzo notes that "the chimeric molecular beacon assay for RNAse H is highly sensitive with fluorescent enhancement of up to 40-fold." (p. 282, conclusions). While the chimeric beacon disclosed by Rizzo was not disclosed in the detection of nucleic acids in a format where RNAse H was present, noting the teaching by Rizzo of a molecular beacon with sensitivity to RNAse H activity, it would have been obvious to one of ordinary skill in the art of NASBA amplification, a method in which RNAse H is often included, to incorporate the molecular beacon disclosed by Rizzo into the method of amplification and detection taught by Ovyn with a reasonable expectation for success.

***Related Prior Art***

5. The prior art made of record and not relied upon is considered pertinent to applicant's disclosure. Yu et al. (WO03/038119; published May 2003) disclose a method of amplification of foot and mouth disease virus (FDMV) using NASBA with detection using chemiluminescence. Rossi et al. (US Patent 5,783,391; July 1998) disclose a method of amplification via cylic amplification using reverse transcriptase and T7 RNA polymerase, however the ribonuclease is RNase A. de Barr et al. (2001, Journal of Clinical Microbiology, p. 1895-1902) disclose a method for isothermal amplification to identify multiple subtypes of HIV-1 using NASBA amplification and molecular beacons.

***Conclusion***

No claims are allowed.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Stephanie K. Mummert, Ph.D. whose telephone number is 571-272-8503. The examiner can normally be reached on M-F, 9:00-5:30.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Gary Benzion can be reached on 571-272-0782. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free).

Stephanie K. Mummert

Stephanie K Mummert, Ph.D.
Examiner
Art Unit 1637

SKM

Gary Benzion

GARY BENZION, PH.D
SUPERVISORY PATENT EXAMINER
TECHNOLOGY CENTER 1600